AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Canceled).

2. (Currently amended): A bioreactor comprising: ~~The bioreactor of claim 1~~

(a) a first substrate having a first surface, an opposite second surface and edges;

(b) a second substrate having a first surface and an opposite second surface, defining a cavity with a bottom surface, wherein the bottom surface is located therebetween the first surface and the second surface, and wherein the first surface of the first substrate is received by the second surface of the second substrate to cover the cavity so as to form a chamber for receiving cells and a liquid medium; and

(c) a port formed between the bottom surface and the first surface of the second substrate with a first opening and an opposite, second opening, wherein the port is in fluid communication with the chamber through the first opening to allow a stream of substance to be introduced into the chamber through the port substantially along a first direction, [[.]]

wherein the second substrate further defines a third opening and an opposite fourth opening adapted for allowing a flow of liquid to be introduced into the chamber through the third opening and away from the chamber through the fourth opening substantially along a second direction, and wherein the second direction is substantially perpendicular to the first direction.

3. (Original): The bioreactor of claim 2, further comprising a biocompatible coating layer applied to the bottom surface of the second substrate.

4. (Original): The bioreactor of claim 3, wherein the biocompatible coating layer comprises

a material that may inhibit cell adhesion to the biocompatible coating layer, enhance cell adhesion to the biocompatible coating layer, or function as a fluorescent marker or indicator of the state of cells.

5. (Original): The bioreactor of claim 3, wherein the first surface of the first substrate and the second surface of the second substrate is spaced such that when a layer of cells grows on the biocompatible coating layer, the flow of liquid can flow in the chamber between the first surface of the first substrate and the layer of cells.

6. (Original): The bioreactor of claim 5, wherein the flow of liquid is controlled so as to provide a known shear force to the layer of cells.

7. (Original): The bioreactor of claim 5, wherein the flow of liquid is controlled so as to provide perfusion and maintenance to the layer of cells.

8. (Original): The bioreactor of claim 5, wherein the cells comprise bacteria.

9. (Original): The bioreactor of claim 5, wherein the cells comprise protozoa.

10. (Original): The bioreactor of claim 5, wherein the cells comprise endothelial cells.

11. (Original): The bioreactor of claim 5, wherein the first surface of the first substrate and the second surface of the second substrate is spaced to further allow at least one cell to migrate above the layer of cells.

12. (Original): The bioreactor of claim 11, wherein the at least one cell to migrate is a cell different from the layer of cells.

13. (Original): The bioreactor of claim 12, wherein the at least one cell to migrate is a cell same as the layer of cells.

14. (Original): The bioreactor of claim 2, further comprising a layer of porous material positioned on the bottom surface of the second substrate.

15. (Original): The bioreactor of claim 14, further comprising a biocompatible coating layer applied to the layer of porous material such that the layer of porous material is between the biocompatible coating layer and the bottom surface of the second substrate.

16. (Original): The bioreactor of claim 3, wherein the biocompatible coating layer comprises a material that may inhibit cell adhesion to the biocompatible coating layer, enhance cell adhesion to the biocompatible coating layer, or function as a fluorescent marker or indicator of the state of cells.

17. (Original): The bioreactor of claim 3, wherein the first surface of the first substrate and the second surface of the second substrate are spaced such that when a layer of cells grows on the biocompatible coating layer, the flow of liquid can flow in the chamber between the first surface of the first substrate and the layer of cells.

18. (Original): The bioreactor of claim 17, wherein the flow of liquid is controlled so as to provide a known shear force to the layer of cells.

19. (Original): The bioreactor of claim 18, wherein the flow of liquid is controlled so as to provide perfusion and maintenance to the layer of cells.

20. (Original): The bioreactor of claim 18, wherein the cells comprise bacteria.

21. (Original): The bioreactor of claim 18, wherein the cells comprise protozoa.

22. (Original): The bioreactor of claim 17, wherein the cells comprise endothelial cells.

23. (Original): The bioreactor of claim 17, wherein the first surface of the first substrate and the second surface of the second substrate are spaced to further allow at least one cell to migrate above the layer of cells.

24. (Original): The bioreactor of claim 23, wherein the at least one cell to migrate is a cell different from the layer of cells.

25. (Original): The bioreactor of claim 24, wherein the at least one cell to migrate is a cell same as the layer of cells.

26. (Original): The bioreactor of claim 14, wherein the layer of porous material comprises collagen.

27. (Original): The bioreactor of claim 14, wherein the layer of porous material comprises an extracellular matrix.

28. (Original): The bioreactor of claim 14, wherein the layer of porous material comprises at least one cell culture scaffold supportive to the layer of cells.

29. (Original): The bioreactor of claim 14, wherein the layer of porous material allows at least one cell to extravasate below the layer of cells.

30. (Currently amended): The bioreactor of claim 2 ~~claim 1~~, wherein the second substrate is fabricated from glass, Mylar, PDMS, silicon, a polymer, a semiconductor, or any combination of them.

31. (Currently amended): The bioreactor of claim 2 ~~claim 1~~, wherein the first substrate is at least partially optically transparent.

32. (Original): The bioreactor of claim 31, further comprising a plurality of posts positioned

between the first surface of the first substrate and the second surface of the second substrate to substantially maintain a predetermined separation between the first surface of the first substrate and the second surface of the second substrate to allow optical detecting of dynamic activities of cells in the chamber.

33. (Original): The bioreactor of claim 32, wherein the dynamic activities of cells in the chamber are detectable through optical detecting means.

34. (Original): The bioreactor of claim 33, wherein the optical detecting means comprises at least one of high-resolution optical microscope and a fluorescence-imaging device.

35. (Original): The bioreactor of claim 32, wherein the plurality of posts are positioned in at least two rows, and wherein each row of posts has at least two posts spaced from each other.

36. (Original): The bioreactor of claim 32, wherein the first substrate and the second substrate are substantially parallel to each other.

37. (Original): The bioreactor of claim 31, further comprising a biocompatible coating layer applied to the first surface of the first substrate.

38. (Original): The bioreactor of claim 37, wherein the biocompatible coating layer comprises a material that may inhibit cell adhesion to the biocompatible coating layer, enhance cell adhesion to the biocompatible coating layer, or function as a fluorescent marker or indicator of the state of cells.

39. (Currently amended): The bioreactor of <u>claim 2</u> ~~claim 1~~, wherein the stream of substance is controlled so as to provide a gradient to the chamber at least around the first opening.

40. (Original): The bioreactor of claim 39, wherein the stream of substance comprises

chemokine.

41. (Original): The bioreactor of claim 40, wherein the stream of substance comprises a substance affecting the growth of cells.

42. (Currently amended): A bioreactor comprising:

(a) ~~a.~~ a first substrate having a first surface, an opposite second surface and edges;

(b) ~~b.~~ a second substrate having a first surface and an opposite second surface, defining a cavity with a bottom surface, wherein the bottom surface is located therebetween the first surface and the second surface, and wherein the first surface of the first substrate is received by the second surface of the second substrate to cover the cavity so as to form a chamber for receiving cells and a liquid medium; and

(c) ~~c.~~ perfusion means in fluid communication with the chamber to allow diffusional exchange of nutrients and metabolic byproducts with the chamber.

43. (Original): The bioreactor of claim 42, further comprising a port formed between the bottom surface and the first surface of the second substrate with a first opening and an opposite, second opening, wherein the port is in fluid communication with the chamber through the first opening to allow a stream of substance to be introduced into the chamber through the port substantially along a first direction.

44. (Original): The bioreactor of claim 43, wherein the second substrate further defines a third opening and an opposite fourth opening adapted for allowing a flow of liquid to be introduced into the chamber through the third opening and away from the chamber through the fourth opening substantially along a second direction, and wherein the second direction is substantially perpendicular to the first direction.

45. (Original): The bioreactor of claim 44, further comprising a biocompatible coating layer applied to the bottom surface of the second substrate.

46. (Original): The bioreactor of claim 45, wherein the biocompatible coating layer comprises a material that may inhibit cell adhesion to the biocompatible coating layer, enhance cell adhesion to the biocompatible coating layer, or function as a fluorescent marker or indicator of the state of cells.

47. (Original): The bioreactor of claim 45, wherein the first surface of the first substrate and the second surface of the second substrate is spaced such that when a layer of cells grows on the biocompatible coating layer, the flow of liquid can flow in the chamber between the first surface of the first substrate and the layer of cells.

48. (Original): The bioreactor of claim 47, wherein the flow of liquid is controlled so as to provide a known shear force to the layer of cells.

49. (Original): The bioreactor of claim 47, wherein the flow of liquid is controlled so as to provide perfusion and maintenance to the layer of cells.

50. (Original): The bioreactor of claim 47, wherein the cells comprise bacteria.

51. (Original): The bioreactor of claim 47, wherein the cells comprise protozoa.

52. (Original): The bioreactor of claim 47, wherein the cells comprise endothelial cells.

53. (Original): The bioreactor of claim 47, wherein the first surface of the first substrate and the second surface of the second substrate is spaced to further allow at least one cell to migrate above the layer of cells.

54. (Original): The bioreactor of claim 53, wherein the at least one cell to migrate is a cell different from the layer of cells.

55. (Original): The bioreactor of claim 54, wherein the at least one cell to migrate is a cell same as the layer of cells.

56. (Original): The bioreactor of claim 44, further comprising a layer of porous material positioned on the bottom surface of the second substrate.

57. (Original): The bioreactor of claim 56, further comprising a biocompatible coating layer applied to the layer of porous material such that the layer of porous material is between the biocompatible coating layer and the bottom surface of the second substrate.

58. (Original): The bioreactor of claim 57, wherein the biocompatible coating layer comprises a material that may inhibit cell adhesion to the biocompatible coating layer, enhance cell adhesion to the biocompatible coating layer, or function as a fluorescent marker or indicator of the state of cells.

59. (Original): The bioreactor of claim 57, wherein the first surface of the first substrate and the second surface of the second substrate is spaced such that when a layer of cells grows on the biocompatible coating layer, the flow of liquid can flow in the chamber between the first surface of the first substrate and the layer of cells.

60. (Original): The bioreactor of claim 59, wherein the flow of liquid is controlled so as to provide a known shear force to the layer of cells.

61. (Original): The bioreactor of claim 60, wherein the flow of liquid is controlled so as to provide perfusion and maintenance to the layer of cells.

62. (Original): The bioreactor of claim 61, wherein the cells comprise bacteria.

63. (Original): The bioreactor of claim 61, wherein the cells comprise protozoa.

64. (Original): The bioreactor of claim 61, wherein the cells comprise endothelial cells.

65. (Original): The bioreactor of claim 61, wherein the first surface of the first substrate and the second surface of the second substrate is spaced to further allow at least one cell to migrate above the layer of cells.

66. (Original): The bioreactor of claim 65, wherein the at least one cell to migrate is a cell different from the layer of cells.

67. (Original): The bioreactor of claim 66, wherein the at least one cell to migrate is a cell same as the layer of cells.

68. (Original): The bioreactor of claim 56, wherein the layer of porous material comprises collagen.

69. (Original): The bioreactor of claim 56, wherein the layer of porous material comprises an extracellular matrix.

70. (Original): The bioreactor of claim 56, wherein the layer of porous material comprises at least one cell culture scaffold supportive to the layer of cells.

71. (Original): The bioreactor of claim 56, wherein the layer of porous material allows at least one cell to extravasate below the layer of cells.

72. (Original): The bioreactor of claim 43, wherein the stream of substance is controlled so as to provide a gradient to the chamber at least around the first opening.

73. (Original): The bioreactor of claim 72, wherein the stream of substance comprises chemokine.

74. (Original): The bioreactor of claim 72, wherein the stream of substance comprises a substance affecting the growth of cells.

75. (Original): The bioreactor of claim 42, wherein the second substrate is fabricated from glass, Mylar, PDMS, silicon, a polymer, a semiconductor, or any combination of them.

76. (Original): The bioreactor of claim 42, wherein the first substrate is at least partially optically transparent.

77. (Original): The bioreactor of claim 76, further comprising a plurality of posts positioned between the first surface of the first substrate and the second surface of the second substrate to substantially maintain a predetermined separation between the first surface of the first substrate and the second surface of the second substrate to allow optical detecting of dynamic activities of cells in the chamber.

78. (Original): The bioreactor of claim 77, wherein the dynamic activities of cells in the chamber are detectable through optical detecting means.

79. (Original): The bioreactor of claim 78, wherein the optical detecting means comprises at least one of high-resolution optical microscope and a fluorescence-imaging device.

80. (Original): The bioreactor of claim 77, wherein the plurality of posts are positioned in at least two rows, and wherein each row of posts has at least two posts spaced from each other.

81. (Original): The bioreactor of claim 77, wherein the first substrate and the second substrate are substantially parallel to each other.

82. (Original): The bioreactor of claim 76, further comprising a biocompatible coating layer applied to the first surface of the first substrate.

83. (Original): The bioreactor of claim 82, wherein the biocompatible coating layer comprises a material that may inhibit cell adhesion to the biocompatible coating layer, enhance cell adhesion to the biocompatible coating layer, or function as a fluorescent marker or indicator of the state of cells.

84. (Currently amended): The bioreactor of claim 42, wherein the perfusion means comprises:

(a) ~~a.~~ a nanofilter with a plurality of pores in fluid communication with the chamber, wherein the pores are sized to allow diffusional exchange of nutrients and metabolic byproducts with the chamber and not to allow cells to migrate across the nanofilter; and

(b) ~~b.~~ a perfusion supply network in fluid communication with the nanofilter through the pores.

85. (Original): The bioreactor of claim 84, wherein the pores are further sized to allow cells to perfuse through only by bi-directional diffusion through the nanofilter in a manner such that substantially no shear is generated by the perfusion of cells.

86. (Currently amended): The bioreactor of claim 85, wherein the perfusion supply network comprises:

(a) ~~a.~~ a plurality of perfusion channels, each being in fluid communication with the nanofilter to allow bi-directional, diffusional exchange of nutrients and metabolic byproducts with the nanofilter and being dimensioned to minimize pressure drops along each perfusion channel and to allow passive diffusional exchange of nutrients and metabolic byproducts along each perfusion channel;

(b) ~~b.~~ a plurality of intermediate supply channels, each being in fluid communication with a plurality of corresponding perfusion channels so as to provide perfusate to the plurality of corresponding perfusion channels; and

(c) ~~c.~~ a plurality of intermediate return channels, each being in fluid communication

with a plurality of corresponding perfusion channels so as to collect perfusate from the plurality of corresponding perfusion channels.

87. (Currently amended): The bioreactor of claim 86, wherein the perfusion supply network further comprises:

(a) ~~a.~~ a plurality of main supply channels, each being in fluid communication with a plurality of corresponding intermediate supply channels so as to provide perfusate to the plurality of corresponding intermediate supply channels; and

(b) ~~b.~~ a plurality of main return channels, each being in fluid communication with a plurality of corresponding intermediate return channels so as to collect perfusate from the plurality of corresponding intermediate return channels.

88. (Original): The bioreactor of claim 84, wherein the pores of the nanofilter are sized to have a dimension smaller than 400 nanometers cross-sectionally.

89. (Currently amended): A bioreactor comprising:

(a) ~~a.~~ a first substrate having a first surface, an opposite second surface and edges;

(b) ~~b.~~ a second substrate having a first surface and an opposite second surface, defining a cavity with a bottom surface, wherein the bottom surface is located therebetween the first surface and the second surface, and wherein the first surface of the first substrate is received by the second surface of the second substrate to cover the cavity so as to form a chamber for receiving cells and a liquid medium;

(c) ~~c.~~ a filter dividing the chamber into a first subchamber and a second subchamber, wherein the filter has a porosity to allow the first subchamber and the second subchamber in fluid communication; and

(d) ~~d.~~ a port formed between the bottom surface and the first surface of the second substrate with a first opening and an opposite, second opening, wherein the port is in fluid communication with the second subchamber through the first opening to allow a stream of substance to be introduced into the chamber through the port

substantially along a first direction.

90. (Original): The bioreactor of claim 89, wherein the second substrate further defines a third opening and an opposite fourth opening adapted for allowing a flow of liquid to be introduced into at least one of the first subchamber and the second subchamber through the third opening and away from at least one of the first subchamber and the second subchamber through the fourth opening substantially along a second direction, and wherein the second direction is substantially perpendicular to the first direction.

91. (Original): The bioreactor of claim 90, wherein the filter has a first surface that defines the first subchamber with the first surface of the first substrate, and an opposite second surface that defines the second subchamber with the second surface of the second substrate.

92. (Original): The bioreactor of claim 91, wherein the filter comprises a perfusion membrane with a plurality of pores in fluid communication with at least one of the first subchamber and the second subchamber, wherein the pores are sized to allow diffusional exchange of nutrients and metabolic byproducts with at least one of the first subchamber and the second subchamber and not to allow cells to migrate across the filter.

93. (Original): The bioreactor of claim 92, wherein the pores are further sized to allow cells to perfuse through only by bi-directional diffusion through the filter in a manner such that substantially no shear is generated by the perfusion of cells.

94. (Original): The bioreactor of claim 93, wherein the pores of the filter are sized to have a dimension smaller than 400 nanometers cross-sectionally.

95. (Original): The bioreactor of claim 91, further comprising a plurality of posts positioned between the first surface of the first substrate and the first surface of the filter to substantially maintain a predetermined separation between the first surface of the first

substrate and the first surface of the filter to allow optical detecting of dynamic activities of cells in the first subchamber.

96. (Original): The bioreactor of claim 95, further comprising a plurality of posts positioned between the second surface of the second substrate and the second surface of the filter to substantially maintain a predetermined separation between the second surface of the second substrate and the second surface of the filter to allow optical detecting of dynamic activities of cells in the second subchamber.

97. (Original): The bioreactor of claim 96, wherein the plurality of posts are positioned in at least two rows, and wherein each row of posts has at least two posts spaced from each other.

98. (Original): The bioreactor of claim 96, wherein when a first flow of liquid is introduced into the first subchamber, the first flow of liquid is controlled so as to provide a known shear force to a first layer of cells growing in the first subchamber on the first surface side of the filter and an environment that simulates a vascular space in the first subchamber.

99. (Original): The bioreactor of claim 98, wherein when a second flow of liquid is introduced into the second subchamber, the second flow of liquid is controlled so as to provide an environment that simulates a tissue space in the second subchamber.

100. (Original): The bioreactor of claim 99, wherein the first flow of liquid and the second flow of liquid are different.

101. (Original): The bioreactor of claim 98, wherein a second layer of cells is capable of growing in the second subchamber on the second surface side of the filter.

102. (Original): The bioreactor of claim 101, wherein the first layer of cells growing in the

first subchamber and the second layer of cells growing in the second subchamber are different.

103. (Original): The bioreactor of claim 90, further comprising an extension port member defining a channel therein, wherein the extension port member is positioned complimentary to the port such that the channel of the extension port member is in fluid communication with the port and the first subchamber to allow the stream of substance is introduced to the first subchamber.

104. (Original): The bioreactor of claim 89, wherein the second substrate is fabricated from glass, Mylar, PDMS, silicon, a polymer, a semiconductor, or any combination of them.

105. (Original): The bioreactor of claim 89, wherein the first substrate is at least partially optically transparent.

106. (Original): The bioreactor of claim 89, wherein the stream of substance is controlled so as to provide a gradient to the chamber at least around the first opening.

107. (Original): The bioreactor of claim 106, wherein the stream of substance comprises chemokine.

108. (Original): The bioreactor of claim 106, wherein the stream of substance comprises a substance affecting the growth of cells.

109. (Currently amended): A bioreactor comprising:

(a) ~~a.~~ a first substrate having a first surface, an opposite second surface and edges;

(b) ~~b.~~ a second substrate having a first surface and an opposite second surface, defining a cavity with a bottom surface, wherein the bottom surface is located therebetween the first surface and the second surface, and wherein the first surface of the first substrate is received by the second surface of the second

substrate to cover the cavity so as to form a chamber for receiving cells and a liquid medium;

(c) ~~e.~~ a first filter dividing the chamber into a first subchamber and a second subchamber, wherein the first filter has a porosity to allow the first subchamber and the second subchamber in fluid communication;

(d) ~~d.~~ perfusion means in fluid communication with at least one of the first subchamber and the second subchamber to allow diffusional exchange of nutrients and metabolic byproducts with the chamber; and

(e) ~~e.~~ a port formed between the bottom surface and the first surface of the second substrate with a first opening and an opposite, second opening, wherein the port is in fluid communication with the second subchamber through the first opening to allow a stream of substance to be introduced into the chamber through the port substantially along a first direction.

110. (Original): The bioreactor of claim 109, further comprising a port formed between the bottom surface and the first surface of the second substrate with a first opening and an opposite, second opening, wherein the port is in fluid communication with the second subchamber through the first opening to allow a stream of substance to be introduced into the chamber through the port substantially along a first direction.

111. (Original): The bioreactor of claim 110, wherein the second substrate further defines a third opening and an opposite fourth opening adapted for allowing a flow of liquid to be introduced into at least one of the first subchamber and the second subchamber through the third opening and away from at least one of the first subchamber and the second subchamber through the fourth opening substantially along a second direction, and wherein the second direction is substantially perpendicular to the first direction.

112. (Original): The bioreactor of claim 109, wherein the first filter has a first surface that defines the first subchamber with the first surface of the first substrate, and an opposite second surface that defines the second subchamber with the second surface of the second

substrate.

113. (Original): The bioreactor of claim 112, wherein the first filter comprises a perfusion membrane with a plurality of pores in fluid communication with at least one of the first subchamber and the second subchamber, wherein the pores are sized to allow diffusional exchange of nutrients and metabolic byproducts with at least one of the first subchamber and the second subchamber and not to allow cells to migrate across the first filter.

114. (Original): The bioreactor of claim 113, wherein the pores are further sized to allow cells to perfuse through only by bi-directional diffusion through the first filter in a manner such that substantially no shear is generated by the perfusion of cells.

115. (Original): The bioreactor of claim 114, wherein the pores of the first filter are sized to have a dimension smaller than 400 nanometers cross-sectionally.

116. (Original): The bioreactor of claim 112, further comprising a plurality of posts positioned between the first surface of the first substrate and the first surface of the first filter to substantially maintain a predetermined separation between the first surface of the first substrate and the first surface of the first filter to allow optical detecting of dynamic activities of cells in the first subchamber.

117. (Original): The bioreactor of claim 116, further comprising a plurality of posts positioned between the second surface of the second substrate and the second surface of the first filter to substantially maintain a predetermined separation between the second surface of the second substrate and the second surface of the first filter to allow optical detecting of dynamic activities of cells in the second subchamber.

118. (Original): The bioreactor of claim 117, wherein the plurality of posts are positioned in at least two rows, and wherein each row of posts has at least two posts spaced from each other.

119. (Original): The bioreactor of claim 117, wherein when a first flow of liquid is introduced into the first subchamber, the first flow of liquid is controlled so as to provide a known shear force to a first layer of cells growing in the first subchamber on the first surface side of the first filter and an environment that simulates a vascular space in the first subchamber.

120. (Original): The bioreactor of claim 119, wherein when a second flow of liquid is introduced into the second subchamber, the second flow of liquid is controlled so as to provide an environment that simulates a tissue space in the second subchamber.

121. (Original): The bioreactor of claim 120, wherein the first flow of liquid and the second flow of liquid are different.

122. (Original): The bioreactor of claim 119, wherein a second layer of cells is capable of growing in the second subchamber on the second surface side of the first filter.

123. (Original): The bioreactor of claim 122, wherein the first layer of cells growing in the first subchamber and the second layer of cells growing in the second subchamber are different.

124. (Currently amended): The bioreactor of claim 109, wherein the perfusion means comprises:

(a) ~~a.~~ a second filter with a plurality of pores in fluid communication with the second subchamber, wherein the pores are sized to allow diffusional exchange of nutrients and metabolic byproducts with the second subchamber and not to allow cells to migrate across the second filter; and

(b) ~~b.~~ a perfusion supply network in fluid communication with the second filter through the pores.

125. (Currently amended): The bioreactor of claim 124, wherein the perfusion supply network comprises:

(a) ~~a.~~ a plurality of perfusion channels, each being in fluid communication with the second filter to allow bi-directional, diffusional exchange of nutrients and metabolic byproducts with the second filter and being dimensioned to minimize pressure drops along each perfusion channel and to allow passive diffusional exchange of nutrients and metabolic byproducts along each perfusion channel;

(b) ~~b.~~ a plurality of intermediate supply channels, each being in fluid communication with a plurality of corresponding perfusion channels so as to provide perfusate to the plurality of corresponding perfusion channels; and

(c) ~~c.~~ a plurality of intermediate return channels, each being in fluid communication with a plurality of corresponding perfusion channels so as to collect perfusate from the plurality of corresponding perfusion channels.

126. (Currently amended): The bioreactor of claim 125, wherein the perfusion supply network further comprises:

(a) ~~a.~~ a plurality of main supply channels, each being in fluid communication with a plurality of corresponding intermediate supply channels so as to provide perfusate to the plurality of corresponding intermediate supply channels; and

(b) ~~b.~~ a plurality of main return channels, each being in fluid communication with a plurality of corresponding intermediate return channels so as to collect perfusate from the plurality of corresponding intermediate return channels.

127. (Original): The bioreactor of claim 124, wherein the pores of the second filter are sized to have a dimension smaller than 400 nanometers cross-sectionally.

128. (Original): The bioreactor of claim 124, wherein the first filter and the second filter are different.

129. (Original): The bioreactor of claim 110, further comprising at least one insertion member

defining a cavity therein, wherein the insertion member has a length L and is positioned through the second substrate such that the cavity of the insertion member is in fluid communication with the first subchamber.

130. (Original): The bioreactor of claim 129, further comprising a plug having a first surface and an opposite second surface and complimentary to a corresponding insertion member such that when the plug is received into the cavity of the corresponding insertion member, the plug engages with the body of the corresponding insertion member to seal the cavity and a volume is formed between the first surface and the first filter to allow a collection of cells to be received therein.

131. (Original): The bioreactor of claim 130, wherein the collection of cells comprises tumor cells.

132. (Original): The bioreactor of claim 131, wherein the plug further defines a port in fluid communication with the volume for injecting or withdrawing a stream of substance affecting the growth of the tumor cells.

133. (Original): The bioreactor of claim 129, further comprising a cage adapted for separating the tumor cells from the first subchamber.

134. (Original): The bioreactor of claim 129, further comprising a plurality of electrodes adapted for electrochemical measurements of the tumor cells.

135. (Original): The bioreactor of claim 109, further comprising an extension port member defining a channel therein, wherein the extension port member is positioned such that the channel of the extension port member is in fluid communication with the first subchamber to allow a stream of substance is introduced to the first subchamber.

136. (Original): The bioreactor of claim 135, wherein the stream of substance is controlled so

as to provide a gradient to the first subchamber.

137. (Original): The bioreactor of claim 136, wherein the stream of substance comprises chemokine.

138. (Original): The bioreactor of claim 136, wherein the stream of substance comprises a substance affecting the growth of cells.

139. (Original): The bioreactor of claim 109, wherein the second substrate is fabricated from glass, Mylar, PDMS, silicon, a polymer, a semiconductor, or any combination of them.

140. (Original): The bioreactor of claim 109, wherein the first substrate is at least partially optically transparent.

141. (Original): The bioreactor of claim 109, wherein the stream of substance is controlled so as to provide a gradient to the second subchamber.

142. (Original): The bioreactor of claim 141, wherein the stream of substance comprises chemokine.

143. (Original): The bioreactor of claim 141, wherein the stream of substance comprises a substance affecting the growth of cells.

144. (Currently amended): A layered perfusion system ~~900~~ for use in a bioreactor, wherein the bioreactor defines a chamber for receiving cells and liquid medium, comprising ~~comprises~~:

(a) ~~a.~~ a filter ~~903~~ having a first surface ~~903a~~ and an opposite, second surface ~~903b~~ and a plurality of pores ~~903c~~ defined therein; and

(b) ~~b.~~ a perfusion supply network in fluid communication with the filter ~~903~~ through the pores ~~903c~~.

145. (Currently amended): The bioreactor of claim 144, wherein the perfusion supply network comprises a first perfusion system layer ~~904~~ having a first surface ~~904a~~ and an opposite, second surface ~~904b~~ and a plurality of perfusion channels ~~904c~~ defined therein, wherein the first surface ~~904a~~ of the first perfusion system layer ~~904~~ is received by the second surface ~~903b~~ of the filter ~~903~~ such that at each of the plurality of perfusion channels ~~904c~~ is in fluid communication with the filter ~~903~~ to allow bi-directional, diffusional exchange of nutrients and metabolic byproducts with the filter ~~903~~ and is dimensioned to minimize pressure drops along each perfusion channel ~~904c~~ and to allow passive diffusional exchange of nutrients and metabolic byproducts along each perfusion channel ~~904c~~.

146. (Currently amended): The bioreactor of claim 145, wherein the perfusion supply network further comprises a second perfusion system layer ~~905~~ having a first surface ~~905a~~ and an opposite, second surface ~~905b~~ and a plurality of perfusion supply and return channels ~~905c~~ defined therein, wherein the first surface ~~905a~~ of the second perfusion system layer ~~904~~ is received by the second surface ~~904b~~ of the first perfusion system layer ~~904~~ such that each of the plurality of perfusion supply and return channels ~~905c~~ is in fluid communication with at least one of the plurality of perfusion channels ~~904c~~, and wherein the plurality of perfusion supply and return channels ~~904c~~ are formed along a direction substantially perpendicular to that of the plurality of perfusion channels ~~904c~~.

147. (Currently amended): The bioreactor of claim 146, wherein the perfusion supply network further comprises a third perfusion system layer ~~906~~ having a first surface ~~906a~~ and an opposite, second surface ~~906b~~ and a plurality of intermediate supply and return channels ~~906c~~ defined therein, wherein the first surface ~~906a~~ of the third perfusion system layer ~~906~~ is received by the second surface ~~905b~~ of the second perfusion system layer ~~905~~ such that each of the plurality of intermediate supply and return channels ~~906c~~ is in fluid communication with at least one of the plurality of perfusion supply and return channels ~~905c~~, and wherein the plurality of intermediate supply and return channels ~~906c~~ are formed along a direction substantially perpendicular to that of the plurality of perfusion

supply and return channels ~~905e~~.

148. (Currently amended): The bioreactor of claim 147, wherein the perfusion supply network further comprises a fourth perfusion system layer ~~907~~ having a first surface ~~907a~~ and an opposite, second surface ~~907b~~ and a plurality of main supply and return channels ~~907c~~ defined therein, wherein the first surface ~~907a~~ of the fourth perfusion system layer ~~907~~ is received by the second surface ~~906b~~ of the third perfusion system layer ~~906~~ such that each of the plurality of main supply and return channels ~~907c~~ is in fluid communication with at least one of the plurality of intermediate supply and return channels ~~906c~~, and wherein the plurality of main supply and return channels ~~907c~~ are formed along a direction substantially perpendicular to that of the plurality of intermediate supply and return channels ~~906c~~.

149. (Currently amended): The bioreactor of claim 148, wherein the perfusion supply network further comprises a fifth perfusion system layer ~~911~~ having a first surface ~~911a~~ and an opposite, second surface ~~911b~~ and a supply channel ~~911c~~ and a return channel ~~911d~~ defined therein, wherein the first surface ~~911a~~ of the fifth perfusion system layer ~~911~~ is received by the second surface ~~907b~~ of the fourth perfusion system layer ~~907~~ such at both of supply and return channels ~~911c, 911d~~ are in fluid communication with at least one of the plurality of main supply and return channels ~~907c~~, and wherein the supply and return channels ~~911c, 911d~~ are formed along a direction substantially perpendicular to that of the plurality of main supply and return channels ~~907c~~.

150. (Currently amended): The bioreactor of claim 149, further comprising a supply port ~~908~~ defining a channel ~~908a~~ in fluid communication with the supply channel ~~911c~~, and a drain port ~~909~~ defining a channel ~~909a~~ in fluid communication with the return channel ~~911d~~.

151. (Currently amended): The bioreactor of claim 144, wherein the filter ~~903~~ is in fluid communication with the chamber of the bioreactor and each of the plurality of perfusion

channels ~~904c~~ is in fluid communication with the filter ~~903~~ to allow bi-directional, diffusional exchange of nutrients and metabolic byproducts with the chamber of the bioreactor through the pores ~~903c~~ of the filter ~~903~~.

152. (Currently amended): The bioreactor of claim 144, wherein the pores ~~903c~~ are sized to allow diffusional exchange of nutrients and metabolic byproducts with the chamber and not to allow cells to migrate across the filter ~~903~~.

153. (Currently amended): The bioreactor of claim 144, wherein the pores ~~903c~~ of the second filter ~~903~~ are sized to have a dimension smaller than 400 nanometers cross-sectionally.

154. (Currently amended): A method for preparing a layered perfusion system ~~900~~ for use in a bioreactor, wherein the bioreactor defines a chamber for receiving cells and liquid medium, comprising ~~comprises~~ the steps of:

(a) ~~a.~~ arranging a silicon wafer ~~953~~, a silicon-nitride layer ~~952~~, and a coblock polymer layer ~~951~~ such that the silicon-nitride layer ~~952~~ is positioned between the silicon wafer ~~953~~ and the coblock polymer layer ~~951~~;

(b) ~~b.~~ etching a plurality of channels ~~904c~~ in the silicon wafer ~~953~~; and

(c) ~~c.~~ etching a plurality of pores ~~903c~~ through the silicon-nitride layer ~~952~~ to form a filter ~~903~~ such that the plurality of pores are in fluid communication with the plurality of channels ~~904c~~.

155. (Currently amended): The bioreactor of claim 154, prior to the step of etching a plurality of pores ~~903c~~, further comprising a step of patterning the coblock polymer layer ~~951~~ to form a plurality of opening corresponding to positions where the plurality of pores ~~903c~~ are to be formed.